“Urinary Biochemical Profile in Urolithiasis:

A case control study from Tamil Nadu”

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A Dissertation submitted to

The Tamil Nadu Dr. M.G.R. Medical University,

Chennai, in partial fulfillment of the requirements for

M.Ch. Branch-IV (Genitourinary Surgery)

examination to be held in August 2008.

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CERTIFICATE

This is to certify that the work incorporated in this dissertation entitled ***"Urinary Biochemical Profile in Urolithiasis: A case control study from Tamil Nadu"*** is bonafide work done by Dr. Gaurav Gupta in the partial fulfillment of the rules and regulation for M.Ch. Branch-IV (Genitourinary Surgery) examination of The Tamil Nadu Dr. M.G.R. Medical University, Chennai, to be held in August 2008.

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INTRODUCTION

Considerable progress has been made in the diagnosis and management of Urolithiasis but advances in our understanding of stone formation have not paralleled this. We still wonder why in a family some form stones while others don't, though they are living in the same environment and consuming the same diet. The precipitating factor and sequence of events that lead to the formation of a kidney stone remains elusive.

The likelihood of forming another stone after the first episode is about 10% at 1 year, 30-40% at 5 year, 50-60% at 10 years and possibly 95-100% at 20 to 25 years.[1-4]

Supersaturation is the driving force behind crystal formation in the kidneys. Many physiologic and metabolic derangements have been implicated in the etiology of renal stone formation, but differentiating among these various causes was difficult until the late 1970s. In 1975 Pak et al.[5] developed a simple test to identify the underlying cause of urolithiasis, which was processed into an ambulatory protocol in 1978.[6] This protocol disclosed a physiologic abnormality in nearly 90% of cases and provided a definitive diagnosis in 95% of the patients.[3,4] This ambulatory instrument made diagnostic separation and classification of urolithiasis more accessible and more practical for all patients. The ability to distinguish among the underlying physiologic disturbances allowed the application of a selective treatment programme on the basis of correction of the specific physiologic derangement. Over the years, selective therapies were formed that contain three different components: high fluid intake, specific diet,

and medication. Although much is understood about physical chemistry involved in Urolithiasis and 24-hour urinary collection is consider integral for the selection of appropriate intervention to prevent kidney stone recurrences, the significance of the urinary profile, magnitude of their clinical effects and efficacy of selective treatment remains to be define.[9-11]

AIM

Aim of the study was, to identify the differences in urinary profile of a stone former and matched member of the same family.

REVIEW OF LITERATURE

Upper urinary tract stones are a significant cause of morbidity and resulting cost of treatment. The natural history of calcium urolithiasis has been difficult to establish. Several early studies addressed the natural history of calcium urolithiasis.[1,2,12,13] The likelihood of forming another stone after the first episode is about 10% at 1 year, 30-40% at 5 year, 50-60% at 10 years and possibly 95-100% at 20 to 25 years.[1-4] Consequently, strategies aimed at stone prevention are attractive.

The goal of clinical and laboratory evaluation of patients with stone disease is to obtain information needed for management of patients presenting with stone and for prevention of further stones in these patients. Likewise, the identification of individuals at high risk of stone formation can improve the selection, evaluation and treatment of patients. On the other hand, the understanding of the mechanisms of idiopathic calcium nephrolithiasis is controversial because we are still unable to establish clear-cut cause-effect relationship between metabolic and physicochemical abnormalities and stone formation. A family history of stone formation substantially increases the risk of stone formation suggesting that diet and life style modifications may be useful in these healthy family members.[14] Encouragingly, the treatment arms of many of the randomized trials have shown dramatic reductions of 50% or more in recurrence rates.[3,15-17] These reductions by medication or dietary interventions emphasize that recurrent stone disease is preventable.

However, surgical treatments, although they remove the offending stone, do little to alter the course of the disease. With the recent advancement of ESWL

and endourological techniques in the surgical management of patients with stone disease there has been marked reduction of the morbidity in the management of stone patients. However there is decreased concern on the part of some practitioners and patients about prevention of stone formation. This is despite the increasing understanding of factors that promote stone growth and an increasing awareness of prevention of stone formation which is within the grasp of a practicing physician.

Given the frequency with which a stone recurs, the development of a medical prophylactic program to prevent stone recurrences is desirable. With proper assessment and management of risk factors, physicians can alleviate stone formation in most patients and avoid expensive urological procedures. Some recommend comprehensive urinary metabolic work-up in recurrent stone formers and limited metabolic workup for the first time stone formers[18,19] and consequent dietary modifications.[20]

2.1. Epidemiology

2.1.1 Prevalance

The lifetime prevalence of kidney stone disease is estimated to be 1% to 15%, with the probability of having a stone varying according to age, gender, race and geographic location.[21] In the United States, the prevalence of stone disease has been estimated to be more than 12% in men and 6% in women.[22,23]

Using data derived from the United States National Health and Nutrition Examination Survey dataset (NHANES II and III), Stamatelou et.al.[24] showed a 5.2% prevalence of kidney stone disease from 1988 to 1994, which was 37%

more than from 1976 to 1980 in which the prevalence was 3.8%. The increase in the prevalence of stone disease has been observed by others also.[25-28] This apparent increase may reflect an actual increase in stone disease or it may stem from increased detection of asymptomatic stones discovered with the greater use and higher sensitivity of imaging studies.[29]

2.1.2. Recurrence rates

Early reports suggested that if left untreated the likelihood of forming another stone after the initial episode was 30% to 40% at 5 years.[30] These figures from observational studies are similar to the recurrence rates in the control arms of recently published randomized trials.[3,17] Many of the randomized trials have shown dramatic reductions of 50% or more in recurrence rates.[3,15-17] These reductions by medication or dietary interventions emphasize that recurrent stone disease is preventable.

2.2. Factors affecting nephrolithiasis and their impact

2.2.1. Non-dietary,

2.2.2. Dietary,

2.2.3. Urinary risk factors,

2.2.4. Renal tubular damage and cellular dysfunction and

2.2.5. Macromolecules

2.2.1. Non-dietary

a) Family history

A family history of stone disease substantially increases the risk of stone formation suggesting that dietary and life style modifications may be useful in

these healthy family members. The risk of becoming a stone former is more than 2.5 times in individuals with a family history of stone disease.[14] A family history increases the risk of kidney stone passage independent of dietary risk factors.[31] This higher risk is likely due to a combination of genetic predisposition as well as similar environmental factors.[29] Identification and characterization of families of recurrent stone formers is essential for the identification of unique genetic, environmental and metabolic factors that predispose individuals to recurrent calcium oxalate stone formation. A polygenic inheritance has been proposed to account for the tendency to calcium oxalate stone formation in families.[32] While a number of genetic factors have been clearly associated with rare forms of nephrolithiasis, the information regarding genetic contribution to the common forms of stone disease is limited.

b) Environmental factors

Seasonal variation in stone disease is likely related to temperature by way of fluid losses through perspiration and perhaps by sunlight-induced increases in vitamin D formation. Individuals working in a hot environment appear to be at a higher risk for stone formation.[33] The highest incidence of stone disease has been noted in the summer months, July through September, with the peak occurring within 1 to 2 months of maximal mean temperatures.[34] In many situations, lack of access to water or toilet facilities may lead to lower fluid intake and with the resulting lower urinary volume there is an increased risk of stone formation. Thus it is likely that climatic and geographical conditions indirectly

influence the prevalence of stone disease through effects on temperature and possibly sunlight.

c) Systemic disorders

Although nephrolithiasis has traditionally been considered a renal disorder, overwhelming evidence suggests that it is in fact a systemic disorder. Primary hyperparathyroidism, renal tubular acidosis, and Crohn's disease are well-described conditions that increase the risk of formation of calcium-containing stones. Primary hyperparathyroidism may be found in 5% of stone formers.[35] More recently, a number of other common conditions have been convincingly linked to nephrolithiasis. Increase in body size as assessed by weight, body mass index (BMI), or waistline measurements is associated with an increase in risk of stone formation independent of other risk factors, including diet.[36] The magnitude of the increase in risk from BMI is higher in women than in men. For example, the risk of stone formation for individuals with a BMI greater than or equal to 30 compared to those with a BMI of 21 to 23 was 30% higher among men but nearly 50% higher among women. Weight gain also increases the risk of stone formation. A 35-lb weight gain since early adulthood increased the risk of stone formation by 40% in men and 80% in women.[29] The mechanism or mechanisms for the increased risk associated with larger body size are unknown.

A history of gout increases the likelihood of forming kidney stones, both uric acid and calcium oxalate. In a national health survey, individuals with gout were 50% more likely to have a history of stones.[37] When examined prospectively, a history of gout was associated with the double risk of stone formation,

independent of diet, weight and medication.[39] Although the mechanism for this relationship is unknown, possibilities include insulin resistance and acid–base defects.

More recently, diabetes mellitus was found to raise the risk of stone formation independent of diet and body size.[40] Cross-sectionally, individuals with a history of type-II diabetes mellitus were 30% more likely to have a history of nephrolithiasis. Prospectively, a history of type-II diabetes mellitus increased the risk of stone formation by 30% to 50% in women but not in men. Irrespective of the aforementioned risk factors some patients need particular attention because of the specific risk factors summarized in Table-1.

Table-1. Specific risk factors for stone formation.[40]

- Start of disease early in life: <25 years
- Stones containing brushite
- Disease associated with stone formation
  - Hyperparathyroidism
  - Renal tubular acidosis (complete/partial)
  - Jejunoileal bypass
  - Crohn's disease
  - Intestinal resection
  - Malabsorptive conditions
  - Sarcoidosis
  - Hyperthyroidism
- Medication associated with stone formation
  - Calcium supplements
  - Vitamin D supplements
  - Ascorbic acid in megadoses (>4 g/day)
  - Sulfonamides † Triamterene † Indinavir †
- Anatomical abnormalities associated with stone formation
  - Tubular ectasia (MSK)
  - PUJ* obstruction
  - Calix diverticulum/calix cyst
  - Ureteral stricture
  - Vesicoureteral reflux
  - Horseshoe kidney
  - Ureterocele

† Noncalcium stones
*Pelvi-ureteral junction

2.2.2. Dietary factors

The epidemic of stones of the upper urinary tract that has swept the developed nations of the world is closely correlated with rising affluence and increasing per capita expenditure on food.[41] Two observations highlight the

association between dietary factors and stone formation. First, the `stone boom', which corresponds to the dramatic increase in the incidence of stone disease in western industrialized nations after World War II, compared to the period during the war when animal protein was poorly consumed and malnutrition was the rule when the incidence of stone disease had declined.[42] Second, the `stone clinic effect', a phenomenon described by the Mayo Clinic years ago to explain the reduction of stone recurrence in 66% of the patients after basic dietary advice.[43]

The composition of the urine is influenced by dietary intake and several dietary factors have been proposed to modify the risk of nephrolithiasis. Nutrients implicated are calcium, animal protein,[44] oxalate,[45] sodium,[46] sucrose,[47] magnesium,[48] and potassium.[49] Patients who develop stones often change their diet. Studies that retrospectively assessed diet may be hampered by recall bias. Other studies have examined the relation between diet and changes in the lithogenic composition of the urine, often using calculated relative supersaturation. However, the composition of the urine does not completely predict risk and not all the components that modify risk are included in the calculation of supersaturation (eg. urine phytate). Thus, prospective studies are best suited for examining the association between dietary factors and risk of actual stone formation.

a) Calcium

Albright F et.al.[50] in 1953, defines the syndrome of idiopathic hypercalciuria consisting of normo-calcemia, low serum phosphorus level and increased urinary calcium excretion in the absence of clearly established causes

of hypercalciuria. Since then, numerous studies have been performed in order to distinguish between increased intestinal calcium absorption (‘‘absorptive’’ hypercalciuria), diminished renal tubular reabsorption of calcium (‘‘renal leak’’ hypercalciuria), or increased bone resorption (‘‘resorptive’’ hypercalciuria) as the primary cause of idiopathic hypercalciuria.[51] Whatever the primary cause, the available data suggests that the syndrome of idiopathic hypercalciuria always includes features of intestinal hyper-absorption and reduced tubular reabsorption of calcium,[52] most likely caused by a mutual imbalance of two calciotropic hormones, that is calcitriol (relatively up-regulated) and parathyroid hormone (relatively down regulated).[53]

Low calcium or high calcium intake— the evidence

Based on the key features of idiopathic hypercalciuria, that is increased intestinal absorption and urinary excretion of calcium, regardless of the physico-chemical meaning of increased urinary calcium, physicians for many decades thought that dietary calcium increases the risk of stone formation.[54]

Although dietary calcium had been strongly suspected of raising the risk of stone disease, men with a higher intake of dietary calcium actually had a lower risk of incidental nephrolithiasis independent of other risk factors. This inverse association has been confirmed in two other prospective studies[55,56] and in an updated analysis.[57] Although the mechanism of this effect is unknown, low calcium intake is known to increase oxalate absorption and urinary excretion and individuals with higher calcium intake have lower 24-hour urine oxalate excretion.[58] Moreover, even if calcium intake is highly restricted (daily calcium

intake 2 mg/kg body weight), a number of patients with idiopathic hypercalciuria still excrete more calcium than they eat and therefore get into negative calcium balance.[59] Indeed, osteopenia has been found in many patients with idiopathic hypercalciuria, and some studies clearly indicate that a low calcium diet contributes to reduced bone mass.[60]

Curhan GC et.al.[14] has shown that low dietary calcium intake may increase the risk of stone formation even among individuals with a family history of stones. The above-mentioned observational data was subsequently confirmed in a randomized trial by Borghi et.al.[3] that compared a low calcium diet (400 mg/d) to a diet containing 1200 mg/d of calcium along with low sodium and low animal protein intake in men with absorptive hypercalciuria type-II and calcium oxalate stones. The calcium oxalate supersaturation declined in all patients over the whole study period but was consistently lower in hypercalciuric male stone formers randomized to a diet restricted in animal protein and salt but with normal calcium content (1200 mg/day) compared with patients consuming a low-calcium (400 mg/day) diet (Figure-1). Interestingly the rate of recurrence fell by 50% in the higher calcium intake group. Unfortunately, the effect of the two study diets on urinary citrate was not elucidated. While some authorities still question whether a high calcium diet reduces the risk of stone formation, overwhelming evidence shows that calcium restriction is not beneficial and may in fact be harmful, both by promoting stone formation and accelerating bone loss.

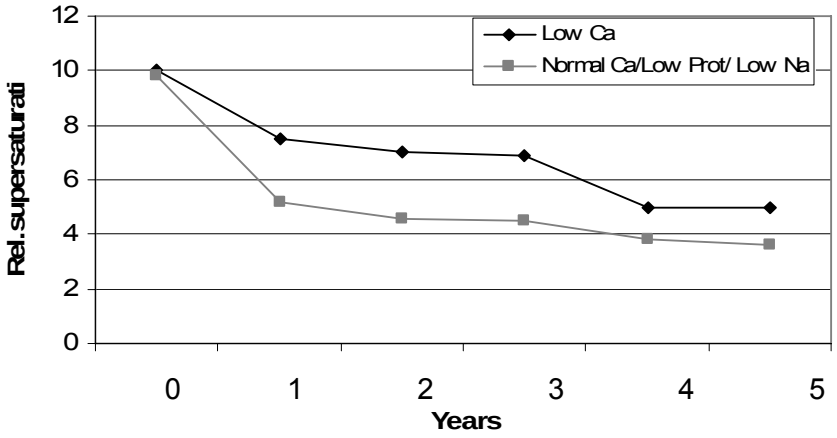

Figure-1: Relative calcium oxalate supersaturation (SSCaOx) of 24 hr urine from male idiopathic calcium stone formers on either normal calcium, low animal protein/low-salt or low-calcium diet. (Redrawn from Borghi L, Schianchi T,Meschi T, et al. Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. N Engl J Med 2002;346;77–84.)

Despite similar bioavailability, the impact of supplemental calcium appears to be different from dietary calcium. In an observational study of older women, calcium supplement users were 20% more likely to form a stone than were women who did not take supplements, after adjusting for dietary factors.[55] In younger women and men there was no association between calcium supplement use and the risk of stone formation.[61,56] The discrepancy between the risks from dietary calcium and the risks from calcium supplements may be due to the timing of calcium intake. In these studies, calcium supplements were often taken between meals, which would diminish binding of dietary oxalate. The recently published Women's Health Initiative randomized trial also demonstrated 17% increase risk of stones with calcium supplementation.[62] However, these results should be interpreted cautiously because the participants were instructed to take

their supplements with meals, and the supplements contained both calcium and vitamin D.

b) Oxalate

The most likely reason why stone recurrences are more frequent on a low calcium diet is reciprocal hyperoxaluria that is increased intestinal oxalate absorption caused by reduced binding of oxalate due to low dietary calcium[63]or mal-absorption syndromes, in which calcium complexion with unabsorbed dietary fat renders dietary oxalate more easily absorbed and leads to hyperoxaluria.[64] Also the alteration in colonic permeability to oxalate (induced by un-absorbable bile salts, also seen in resection of terminal small bowel) have been proposed to explain the intestinal hyper-absorption of oxalate in patients with intestinal hyperoxaluria.[65] The role of dietary oxalate in the pathogenesis of calcium oxalate nephrolithiasis is unclear.[66] The proportion of urinary oxalate derived from dietary oxalate is controversial; estimates range from 10% to 50%.[66] In addition to the gastrointestinal absorption of dietary oxalate, urinary oxalate is also derived from the endogenous metabolism of glycine, glycolate, hydroxyproline, and vitamin C.[29] Due to variable and often low bioavailability, much of the oxalate in food may not be readily absorbed. Bioavailability of oxalate in most foodstuffs is very low (0.01mmol/100g),[67] so practically dietary intake of excessive oxalate is relatively uncommon even in stone formers.[42] Nonetheless, studies have shown that the dietary contribution of urinary oxalate is higher in recurrent calcium oxalate stone formers.[64] Up to one third of patients with calcium oxalate nephrolithiasis may have increased absorption of dietary

oxalate.[68] In some cases, a deficiency of oxalate degradation by the bacterium Oxalobacter formigenes in the gut could be the culprit.[66] In a short-term study in healthy volunteers on a highly standardized dietary regimen (controlled for total calories and intakes of fluid, protein, salt, calcium, and oxalate), the author demonstrated that a 20-fold normal oxalate load (2220 mg/day) induces significant hyperoxaluria (average 69 mg/day) and in some subjects even passage of calcium oxalate crystal aggregates when the diet contained 1200 mg of calcium per day. Average urinary calcium amounted to 128 mg per day.[69] When applying the protocol in the same subjects with the identical oxalate load, but with 3850 mg/day of calcium, hyperoxaluria disappeared (average 29 mg/day) and urinary calcium increased (average 291 mg/day), but no crystal aggregates were passed.[69] This indicates that increases in urinary oxalate carries an increased risk for crystal aggregation of calcium oxalate than increases in urinary calcium even in non-stone formers. Very large amounts of oxalate can be tolerated if they are bound at the intestinal level by a high amount of calcium ingested simultaneously.[69] These data were confirmed by Holmes et.al.[70] who found that the bioavailability of oxalate and urinary oxalate excretion were reduced by increasing the diet's calcium content. In their most recent study, Borghi et. al.[3] also found that urinary oxalate decreased, by an average of 80 µmol per day, on a normal calcium/low animal protein/low salt diet, compared with a low-calcium diet that increased urinary oxalate by an average of 60 µmol per day. Holmes et.al.[70] also demonstrated that dietary oxalate makes a much larger contribution to urinary oxalate excretion than previously recognized,

reaching an average of 53% on a diet containing 250 mg of oxalate and 391 mg of calcium per day. The problems to advise an oxalate-restricted diet for calcium stone patients are

1. Oxalate is found in many foods, thus a low-oxalate regimen will not be palatable or acceptable to all patients.[70]
2. Measurements of the oxalate content of foods vary considerably because of analytical differences and/or uncertainties.[71]
3. Considerable variation of oxalate absorption exists between individuals.[70]

It therefore appears more appropriate to first advice patients on a sufficient calcium intake simultaneously ingested with food in order to avoid increases in urinary oxalate excretion. Nevertheless, efforts to restrict dietary oxalate should be made because they also can be sufficiently effective to limit the degree of calcium-oxalate interaction in the bowel. Though, the value of reducing dietary oxalate intake in order to lower urinary oxalate excretion has been questioned, a recent study demonstrated that urinary oxalate does not increase even on a low calcium diet, when oxalate is restricted simultaneously.[73] The utility of dietary modification is supported by the observation that no pharmacological therapy has been consistently successful in reducing urinary oxalate excretion. The dietary modification is particularly effective in patients who are ingesting a self-selected high dietary oxalate.[42]

The impact of dietary oxalate on risk of stone formation has not yet been studied prospectively because of the lack of sufficient and reliable information on the oxalate content of many foods. However, recent reports using modern

approaches to measure the oxalate content of food[73,74] have opened the possibility of these studies.

c) Protein

Anderson et.al.[75] had showed strong correlation between affluence and nephrolithiasis. Lower urinary tract stones seen in various countries in Southeast Asia were known to be related to protein malnutrition. Due to high dietary protein intake an increased risk of upper tract nepholithiasis has been noted in affluent societies.[75] The relationships among affluence, diet and stone were particularly very impressive because they could be demonstrated within single nations as well as across heterogeneous societies.[76] For example, in northern and western regions of India, animal protein intake is approximately 100% greater than the southern and eastern regions. The risk of upper tract stone formation as estimated from hospitalization rates was 23.9/1000 admissions in north and west compared with 5.9/1000 admissions in the south and east.[42]

Effect of diet protein in clinical studies

Lithogenic effect of protein diet is not established in all instances. Some studies have failed to show that there is a difference in dietary intake between stone formers and controls.[77] In a large study Wasserstein and colleague[78] found that patients with recurrent nephrolithiasis consumed a diet that was similar in its composition to that of a large population of case controls. While there was a clear linear relationship between the dietary protein intake as estimated by excretion of urinary urea nitrogen and urinary calcium, the nature of this relationship suggested that patients with recurrent nephrolithiasis are more sensitive to the

calciuric action of protein. This was because any increment in dietary protein elicited a proportionately greater increase in calciuria in the patients with recurrent nephrolithiasis compared with controls (Figure-2). The underlying mechanism whereby dietary protein leads to hyper-calciuria and the reason for the specific increased sensitivity of patients with recurrent nephrolithiasis to the hypercalciuric action of dietary protein remains unknown. The effect may be due to high protein intake of animal origin contributing to hyperuricosuria due to the purine overload, to hyperoxaluria due to the higher oxalate synthesis, increased acidity of the urine which directly inhibits the calcium absorption from the distal nephron and to hypocitraturia due to the higher tubular reabsorption of citrate due to low urinary pH.[79,80] The citrate chelates urinary calcium, reducing the level of urinary ionized calcium, and citrate may inhibit calcium oxalate crystal growth.[81]

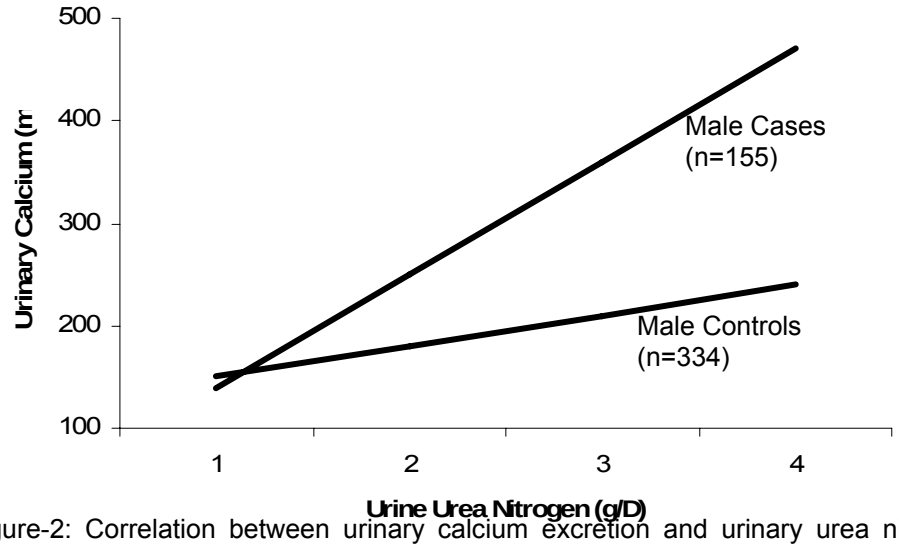

Figure-2: Correlation between urinary calcium excretion and urinary urea nitrogen (an index of dietary protein intake) in patients and controls. The greater slope in the cases versus the controls suggests a greater sensitivity to the calciuric effect of protein in the cases. (Redrawn from: Wasserstein AG, Stolley PD, Soper KA. et.al. Case control stuey of risk factors for idiopathic calcium nephrolithiasis. Miner. Electrolyte.Metab.1987;13:85.)

Wasserstein et.al.[78] had shown that the restriction of dietary protein in 18 patients with recurrent calcium stone formation from 96±5 to 60±65 g/day resulted in significant rise in urinary citrate excretion, from 450±44 to 565±51 mg/day. In the subgroup of hypercalciuric patients (n=11), reduction of dietary protein from 101±4 to 60 to 65 g/day produced even more marked elevation of citrate excretion (451±49 to 632±58 mg/day). Additionally, protein-induced hypercalciuria may be caused by higher bone resorption and lower tubular calcium reabsorption to buffer the acid load, and also by the elevated filtered load of calcium and by the presence of non-reabsorbable calcium sulfate in the tubular lumen.[78] An acute moderate protein restriction reduces urinary oxalate, phosphate, hydroxyproline, calcium and uric acid and increases citrate excretion as recently reported.[82] In population whose intake of dietary protein is reduced or absent such as vegetarians, the risk of nephrolithiasis is markedly reduced.[42] In studies carried out in Great Britain, the prevalence of stone formation was threefold less in a group of vegetarians than in the general population. This protective effect of vegetarianism is present even though increased dietary oxalate may result from high oxalate vegetable intake.[83]

d) Citrate

Low urinary citrate excretion is an accepted risk factor for calcium nephrolithiasis because it reduces urinary supersaturation by complexing calcium ions. In addition, it inhibits growth and aggregation of calcium oxalate and calcium phosphate crystals.[84] By complexing calcium ions, citrate also enhances

the inhibitory activity of Tamm-Horsfall glycoprotein towards calcium oxalate crystal aggregation.[85,86]

Accepted risk factors for hypocitraturia (<1.70 mmol/day) are distal renal tubular acidosis (complete or incomplete) as cytosolic acidification enhances the uptake of citrate from proximal convoluted tubule,[87] acquired metabolic acidosis caused by chronic diarrhoeal states, intracellular acidosis caused by thiazide induced potassium depletion, high sodium intake which induces mild metabolic acidosis caused by increased bi-carbonaturia, excess intake of animal protein (sulfur-containing amino acids), decreased intake of alkali, and primary gastrointestinal mal-absorption of citrate[84] In a study of male patients with recurrent idiopathic calcium nephrolithiasis undergoing 3-day ammonium chloride loading while under free-choice diet, Hess et.al.[88] found that 33% had either low citraturia (<2.12 mmol/day) or overt hypocitraturia (<1.70 mmol/day). Incomplete renal tubular acidosis occurred in 91% and gastrointestinal alkali absorption less than 15 mEq per day in 18% of these patients. In addition, high urinary citrate excretion was correlated with more dietary vegetable fibers, a source of alkali and with urine volume.[88] The latter correlation is possibly because proton secretion along the proximal tubule is flow-dependent, and thus low urine flow would induce mild metabolic acidosis and diminish urinary citrate excretion.[82] On the other hand, administration of bicarbonate-rich mineral water[89] and orange juice[90] increases urinary citrate excretion. Unfortunately, no urinary citrate measurements are available from the two prospective randomized controlled trials on dietary treatment of calcium nephrolithiasis.[5,91]

e) Other nutrients

1. Role of Dietary Sodium in the pathogenesis of Hypercalciuria

There is a close relationship between renal tubular calcium and sodium handling; most factors that promote natriuresis tend to stimulate urinary calcium excretion.[92] Calcium reabsorption parallels the sodium in the renal tubule.[93] The only means to produce a sustained increase in urinary sodium excretion is to increase sodium intake. Dietary sodium may also be a risk factor for hypercalciuria and therefore for calcium oxalate nephrolithiasis. Every 100 mmol increase in dietary sodium increases urinary calcium excretion by 25 mg.[94] The adverse effects of a high NaCl intake and the resultant higher calcium excretion have been well documented by many investigators.[46,95,96] In a previous analysis, multiple regression suggested that a high NaCl intake (16 g/day) was the single variable that was predictive of risk of low bone mineral density in 85 calcium stone forming patients (odds ratio: 3.8) after adjustments for age, weight, body mass index, duration of stone disease, calcium and protein intakes and urinary calcium citrate and uric acid.[97] A high NaCl intake is expected to lower citrate excretion as well.[98] Frusemide and Bumetanide administration produces concomitant calciuria and natriuresis by inhibiting calcium and sodium transport in the thick ascending limb of Henle's loop.[97] Lau et.al.[100] has showen in patients with idiopathic hypercalciuria, the renal hypercalciuric ingested 50 mEq/day more sodium than the diet-dependent hypercalciuria patients. Restriction of dietary sodium intake produces a predicable fall in urinary calcium excretion and lead to the re-categorization of patients with so-called renal-hypercalciuria to diet-

dependent hypercalciuria. It leads to the proposal that modification of the dietary sodium intake may be a rational form of therapy in these patients. The hypercalciuric action of dietary sodium is markedly seen in recurrent calcium stone formers.[42]

Controlled studies failed to show that the general population of patients with recurrent calcium oxalate nephrolithiasis ingests greater mean dietary sodium than controls.[77] It also reinforces the idea that undue sensitivity to calciuric stimuli is present in patients with recurrent calcium stone formers.[42]

2. Vitamin C

The effect of large doses of vitamin C in increasing urinary oxalate excretion is controversial.[101,102] Vitamin C (ascorbic acid) can be metabolized to oxalate; thus higher vitamin C intake could increase the risk of calcium oxalate stone formation. Curhan GC et.al.[103] has showen that the intake of vitamin C was not associated with risk of kidney stones in women. Traxer OA et.al[104] has demonstrated that the consumption of 1000 mg of supplemental vitamin C twice daily increases urinary oxalate excretion by 22%. An observational study in men found that those who consumed ≥1000 mg/day of vitamin C had a 40% higher risk of stone formation compared with men who consumed less than 90 mg/day (the recommended dietary allowance).[57] This relationship was observed only after accounting for dietary potassium intake. Although restricting dietary vitamin C does not seem appropriate (as foods high in vitamin C are also high in inhibitory factors such as potassium), a calcium oxalate stone former should be encouraged to avoid vitamin C supplements.

3. Vitamin B6

Vitamin B6 is a cofactor in oxalate metabolism and vitamin B6 deficiency increases oxalate production and urinary oxalate excretion. Although high doses of supplemental vitamin B6 may be beneficial in selected patients with type-1 primary hyperoxaluria, the use of vitamin B6 in other settings remains unclear. Based on observational data, high intake of vitamin B6 may reduce the risk of kidney stone formation in women[103] but not in men.[105]

Numerous other factors have been studied and implicated in the development of stone formation but many of the associations with risk varies with by age, sex, or BMI. Taylor EN et.al.[57] has observed an increased risk of stone formation among men with higher intake of animal protein and BMI less than 25.

4. Potassium

An epidemiological study has reported that lower the potassium intake, (≤74 mmol/day) higher the relative risk of stone formation[61] whereas potassium supplementation decreases calcium excretion[49] and many potassium-rich foods increase urinary citrate due to their alkali content. Such an effect can be ascribed to an increase in urinary calcium and a decrease in urinary citrate excretion induced by a low potassium intake.[49] Recently, phytate was also found to reduce substantially the likelihood of stone formation in younger women.[56]

5. Magnesium

Magnesium complexes with oxalate, thereby potentially reducing oxalate absorption in the gastrointestinal tract and decreasing calcium oxalate supersaturation in the urine. A few randomized trials have examined the effect of

magnesium supplementation on stone recurrence. However, magnesium was given in combination with other compounds (eg, thiazide diuretic or potassium citrate) and the dropout rates were high. Currently, it is uncertain whether magnesium supplementation has an independent beneficial effect. In prospective observational studies, higher dietary magnesium was associated with a 30% lower risk of stone formation in men,[57] but not in women.[55,56]

6. Fluid intake and beverages

When the urine output is less than 1 L/d, risk of stone formation is markedly higher (Figure-3).[105]

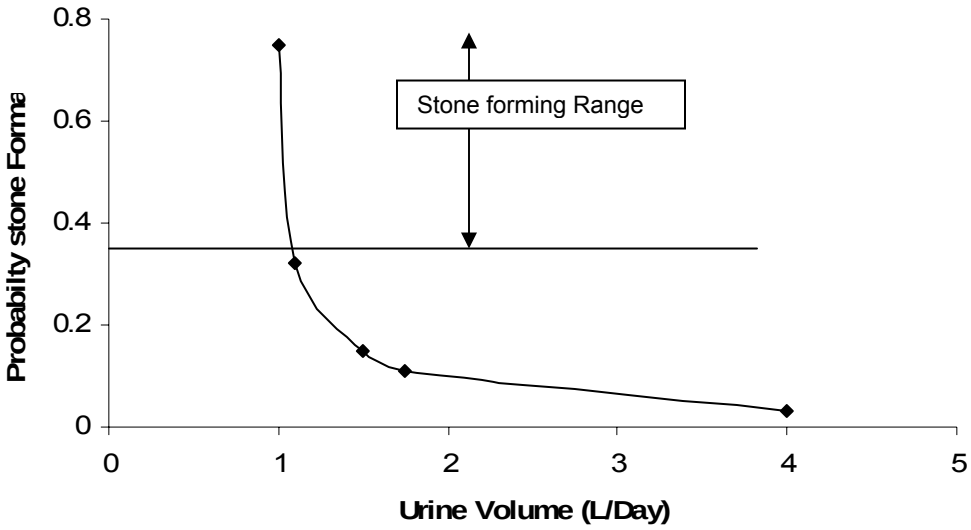

Figure-3: Relationship between the relative probability of stone formation based on analysis of urinary constituents and the 24 hour urinary volume in normal individuals. (Redrawn from: Frank M, DeVries A, Lazebnik J et.al. Epidemilogical investigations of Uroloithiasis in Israel. J Urol;81:497-504:1959.)

Observational studies[55,56,61] and a randomized controlled trial[107] have demonstrated the importance of fluid intake in reducing the likelihood of stone formation. A high fluid intake is a very important goal to reduce urine supersaturation. In a large prospective study of men, who had no history of kidney stones, an inverse association between fluid intake and the risk of urinary

stone formation was observed during 4 years of follow-up.[61] After adjustment for other potentially confounding variables in the multivariate analysis, the relative risk for men decreased significantly from 1.0 in the lowest quintile (<1,275 ml/ day fluid intake) to 0.71 in the highest quintile (>2,538 ml/day fluid intake).[61] This finding in men was consistent with the results of a long-term prospective study among women where the risk of stone formation was inversely related to fluid intake.[55] In the multivariate model, the relative risk for women with the highest fluid intake was 0.61 as compared with those with the lowest intake (1.0), a 39% reduction in risk. In a prospective randomized control trial involving first stone episode patients who were randomized to increase water intake to at least 2 lt/day has shown lower rates of recurrence (12%) compared to those without (27%) and the time interval until the recurrences occurred was significantly longer in patients on high water intake. In this study patients were not on any drug therapy or dietary change, so that the effect was exclusively explained by the selective increase in urinary volume.[107] The study confirms that an increase in fluid intake to assure a consistent urinary volume of at least 2 l/day is the initial therapy for the prevention of stone recurrences.

To what extent the hardness and mineral composition of water affects stone formation remains controversial.[108-110] As the calcium content of drinking water increases, calcium excretion increases but oxalate excretion falls[109,111] Water with a large amount of bicarbonate may increase citrate excretion[109] and magnesium content may favourably alter citrate and magnesium excretion.[112] Based on these findings, there is still no definite evidence that hard water rich in calcium and

magnesium, is more lithogenic than soft water. Fluid therapy could be harmful by dilution of urinary inhibitors with a high urine volume that may paradoxically increase the risk of stone formation.[42] However studies of the formation product ratio and the activity product ratio of urine diluted in-vitro and in-vivo failed to demonstrate an increase in the risk of stone formation with increasing urinary dilution.[113]

Despite previous beliefs to the contrary, observational studies have found that Caffeinated, decaffeinated coffee, tea (risk reduction of 8-10%) beer, and wine (risk reduction of 59%) are associated with a reduced risk of stone formation. [114,115] It was speculated that the protective effects of coffee, tea and wine were caused by urinary dilution, determined by the ability of caffeine and alcohol to inhibit antidiuretic hormone. Therefore, the decreased risk for decaffeinated coffee might have been conferred by another mechanism. Although citrus juices theoretically could reduce the risk of stone formation,[116] orange juice consumption was not associated with stone formation and grapefruit juice intake was associated with a 44% higher risk.[114,115] Grapefruit juice is known to have a number of effects on intestinal enzymes but the mechanism for the observed increased risk is unknown.[29] Previously studies had suggested an increased risk for soda consumption and unadjusted results from observational studies also suggested an increased risk. However, after controlling for other dietary components, consumption of soda (including soda with caffeine, soda without caffeine, diet soda and conventional sweet soda) was not associated with the risk of stone formation.[114,115] Although skim and whole milk were not associated with

risk in the observational studies, probably because these studies adjusted for the intake of dietary calcium, milk intake likely reduces the risk of calcium kidney stone formation. In summary, these results must still be interpreted with caution until adequate long-term randomized trials of dietary interventions are performed.

2.2.3. Urinary factors: The principle of supersaturation

Supersaturation is the driving force behind crystal formation. When the solution is supersaturated with lithogenic substance crystallization occurs and thus nucleation, crystal growth, crystal agglomeration and stone growth. The 24-hour urine chemistries provide important information about uro-metabolic abnormalities and thus direct therapeutic recommendations for prevention.

Traditionally, urine results have been categorized as ‘‘normal’’ or ‘‘abnormal’’ and potential diagnosis from a metabolic evaluation of a calcium stone former are shown in Table-2.

Table-2: Abnormalities from uro-metabolic evaluation of calcium stone former [117]

• Low urinary volume (<2 L/d) • Hyperoxalauria (>40 mg/d) • Hyperuricosuria (>750 mg/d) • Hypocitraturia (<300 mg/d)	• Hypercalciuria (>250 mg/d in women; >300 mg/d in men) o Types I, II, and III absorptive hypercalciuria o Renal leak hypercalciuria o Resorptive hypercalciuria • Hypomagnesuria <3mmol/L

Recently Curhan[29] has emphasized two important points.

1. The urine values are continuous variables so the ‘‘normal’’ or ‘‘abnormal’’ categorization is arbitrary.

2. Stone formation is a disorder of concentration, not just the absolute amount excreted.

In 1978, Pak et al.[2] developed an ambulatory protocol to identify the underlying cause of Urolithiasis. This protocol provided a definitive diagnosis in 95% of the patients.[3,4] This ambulatory instrument made diagnostic separation and classification of urolithiasis more accessible and more practical for all patients. The ability to distinguish among the underlying physiologic disturbances allowed the application of a selective treatment programme on the basis of correction of the specific physiologic derangement. Over the years, selective therapies were formed that contain three different components: high fluid intake, specific diet, and medication.

2.2.3.(1). Solubility and supersaturation

There is a maximum to the amount of a compound that can be kept in stable solution (at equilibrium). This is defined by its solubility or equilibrium concentration product.[118] When the amount in solution exceeds the solubility, supersaturation results. There is a drive to remove the excess by crystallization. This drive can be manipulated in two ways: changing the pool available for precipitation and changing the solubility.[118]

2.2.3.(2). Crystallization as basis of Urolithiasis

Urinary stones are composed of crystalline material usually associated with a matrix of organic material. Irrespective of the type of stone, crystallization is a first step in stone formation. The solubility of a salt depends upon its crystal characteristics and its activity in solution. The first depend on the crystal structure, crystal-solute interactions and crystal size. Small crystals are less stable and their solubility is increased.[118] Below the critical nucleus size of 0.1

μm, crystals are unstable and they prefer to dissolve rather then grow further. (Kelvin or Ostwald-Freundlich effect).[119] The rate at which particles of this size form and survive is the nucleation rate. Stabilization of nuclei by adherence to a different solid phase (heterogeneous nucleation)[6] and de-stabilization by adherence with the substances (inhibition of nucleation) strongly influence the nucleation rate in urine. The available pool is also affected by chelation and changing protonation state (pH effect).[119]

For compounds that have an acid dissociation constant, pKa, within the pH range for urine, 5 to 8, the protonation state can vary. Thus uric acid (pKa 5.35) is prone to precipitate in acidic urine and is more soluble in alkaline urine. Changes in pH also affect other urine compounds. In alkaline urine, citrate is more in its triple de-protonated form, which is more effective in preventing crystallization.[119]

2.2.4. Renal tubular damage and cellular dysfunction

Super-saturation is the driving force behind crystal formation. It can, however, result only in the formation of crystals which can be harmlessly expelled. For stone formation, crystals should be formed and retained in the kidney which is a rare occurrence.[120,121] Crystalluria is common while stone formation is not. The retention risk depends on crystal size and surface, flow dynamics, urinary tract-dimensions and cell-surface characteristics. Lethal epithelial cellular injury promotes crystal nucleation, aggregation and retention. Sub-lethal injury or dysfunctional cells may produce ineffective crystallization modulators and localized areas of supersaturation in the interstitium (Figure-4).

The former will affect crystallization in the urine while the latter may cause precipitation in the interstitium and development of Randall's plaques.[122] He suggested that interstitial sub-epithelial deposits of calcium phosphate or calcium carbonate arising from pathological conditions of the renal papilla eroded through the papillary surface forming a type-I lesion. He further suggested that excessive urinary supersaturation in association with tubular cell death resulted in crystal deposition in the collecting ducts producing a type-II lesion. Both types of lesions acted as foci for further stone growth in the pelvis or papillary ducts.[123] Randall proposed a theory in which both urinary supersaturation and renal tubular damage play a part in stone formation.

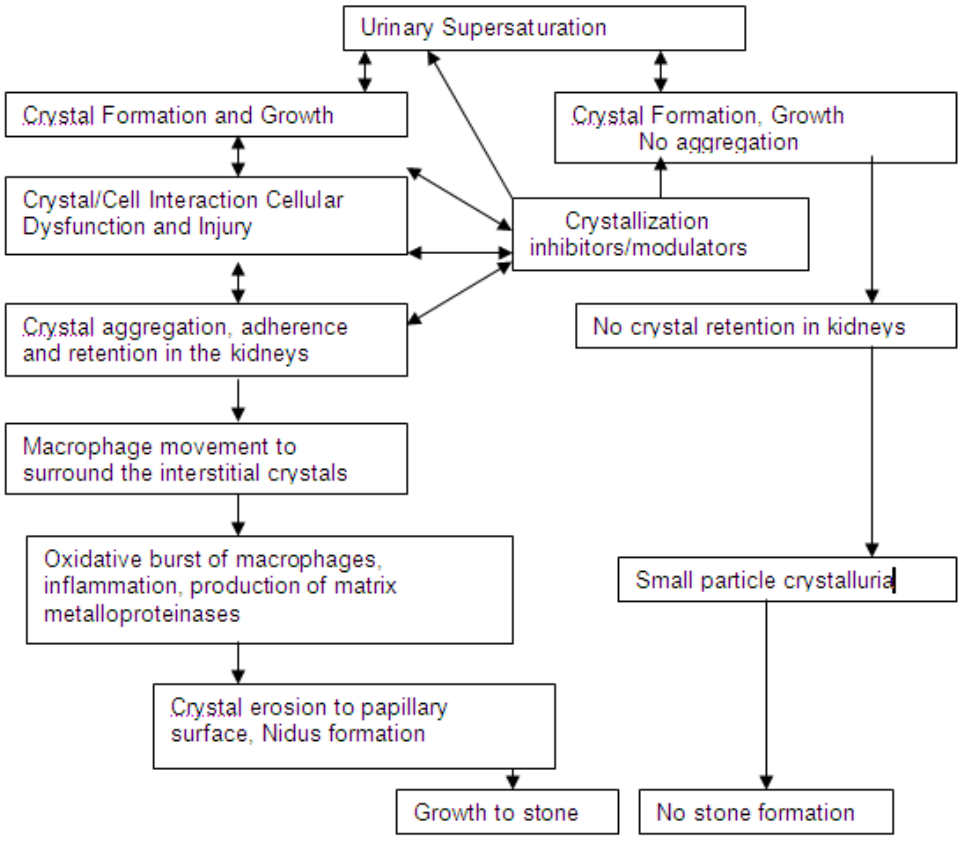

Figure-4: Schematic presentation of relationship between various factors, which lead to formation of idiopathic renal stones. Crystals that are not expelled with the urine induce production of crystallization modulators and may eventually lead to cellular dysfunction and degradation and thus hetrogenous nucleation. Cell injury also promotes interstial inflammation, which is likely involved in crystal erosion to papillary surface and thus stone nidus. (Redrawn from Khan SR.Renal tubular damage/dysfunction: key to the formation of kidney stones Urol. Res. 2006;34: 86–91)

2.2.5. Role of macromolecules

The potential role of intra-crystalline proteins and lipids (macromolecules) in stone genesis has prompted studies to determine whether Tamm–Horsfall glycoprotein (most abundant protein in healthy human urine) is part of the organic matrix of CaOx crystals. Proponents argue that macromolecules are the real key molecules for stone inhibition.[124]

A macromolecule containing multiple calcium binding sites will more strongly bind to growth sites where the density of calcium ions available for binding is greater.[125] Urine contains numerous compounds like citrate, magnesium and macromolecules that can inhibit crystal growth. In whole urine macromolecules with the highest affinity for the growth sites contribute most to the growth inhibition. Tamm-Horsfall and nephrocalcin are potent inhibitor of calcium oxalate monohydrate crystal aggregation, but not growth.[126] Osteopontin and uropontin have been shown to inhibit nucleation, growth, and aggregation of calcium oxalate crystals as well as to reduce binding of crystals to renal epithelial cells in vitro.[127]

MATERIALS AND METHODS

This study was conducted from April 2006 to January 2008 after taking approval from the institutional review board.

3.1. Design of the study

This was a prospective matched Case-Control study. Informed consent (Annexure-i) for recruitment was obtained after a complete urological history (Annexure-ii) followed by serum and urinary examination. Seventy-eight subjects were recruited for the study (39 cases and 39 matched controls). Only patients from Tamilnadu were included in the study.

3.2. Selection criteria

3.2.1. Inclusion criteria for cases

- Eighteen years or older in age,
- Patients who had renal or ureteric stones with normal renal function.

3.2.2. Exclusion criteria for cases

- Patients with bladder stone,
- Endocrine abnormality,
- Anatomic urinary tract abnormality associated with stone formation,
- History of bowel disease or resection predisposing to stone formation,
- Medication and diseases associated with stone formation.

3.2.3. Inclusion criteria for controls

- Eighteen years or older in age,
- Same gender as of the patient,
- First degree relation with the patient,

- Diet matched relative–at least consuming two out of three major meals together,
- No nephrolithiasis or history of stone disease,
- Living in the family with the patient in the same house for at least last 5 years.

Dietary matching was done with the help of the Dietary Department.

3.2.4. Exclusion criteria for controls

- Those with urolithiasis or history of urinary stone disease,
- Endocrine abnormality associated with stone formation,
- History of bowel disease or resection predisposing to stone formation,
- Medication and diseases associated with stone formation.

3.3. Parameters studied

3.3.1. Serum analysis

1. Calcium,
2. Phosphorus,
3. Uric acid,
4. Albumin–globulin ratio,
5. Sodium,
6. Potassium and
7. Bicarbonate.

3.3.2. Urinary analysis

1. Calcium,
2. Oxalate,
3. Uric acid,
4. Citrate,
5. Magnesium,
6. Creatinine,
7. Urinary volume and
8. Urinary pH.

24 hour urinary analysis

3.3.3. Presence of stone disease in controls was ruled out by X-ray and ultrasound of the kidney-ureter-bladder region.

3.4. Urinary collection

The bladder was emptied at 6 AM. All the urine voided after that for the next 24 hours including the 6 AM void of the next day was collected. This procedure was explained to each case and control in detail to ensure proper collection of urine. Ambulatory 24-hour urinary analysis was done on two consecutive days. The first 24-hour urinary collection for analysis of urinary calcium and oxalate was done in a container with 10 ml of 6mmol of hydrochloric acid to prevent precipitation of calcium and oxalate salts and also prevents oxidation of ascorbic acid to oxalate. The second day collection, for urinary pH, uric acid, citrate, magnesium and creatinine was done in a container with 10 ml of 0.3mmol of sodium-azide, to prevent bacterial growth. Urinary volume was calculated averaging the urinary volume collected on the two consecutive days.

There was no dietary restriction and 24-hour urinary samples were collected while on their regular diet at home. Urinary 24-hour sample collection was done before any intervention for urolithiasis.

Table-3 shows the laboratory values for the various test parameters, which were obtained from our institutional nomogram. Urinary abnormalities were hypercalciuria, hyperoxaluria, hyperuricosuria, hypocitraturia and hypomagnesuria.

Table-3: Laboratory values for serum and urinary parameters.

Ambulatory normal serum values	**Values**
Calcium	8.3-10.2 mg/dl
Phosphorus	2.5-4.6 mg/dl
Uric acid	4.0-7.0 mg/dl
Albumin-globulin ration	1.0-1.8
Sodium	135-145 mEq/L
Potassium	3.5-5.0 mEq/L
Bicarbonate	22-29 mmol/L
Ambulatory 24-hr urinary values	**Values**
Low urinary volume	≤ 1500 ml
Hypercalciuria	>300mg for male >250mg for females
Hyperoxaluria	>40mg
Hyperuricosuria	>700 mg
Hypocitraturia	<250 mg
Hypomagnesuria	<3mmol
Urinary creatinine	1-2 gm
Urinary pH	5.5-6.5

3.5. Statistical analysis

Data was entered in a spreadsheet, MS EXCEL and analyzed using Statistical Package for the Social Sciences, version 15.0 (SPSS 15.0) software. All continuous variables were summarized using mean and standard deviation,

while the categorical variables were summarized using frequencies and percentages. The value of each urinary constituent was compared using Mc-Nemar test, to test the difference between cases and their matched controls.

Mc-Nemar test is used to analyze Case-Controls studies, where each case and control is matched. Here we assess matched pairs with respect to one dichotomous variable. If there is no association between the risk factor and the disease, we would expect the number of pairs where cases are exposed to the risk factor but control is not to equal the number of pairs where the controls are exposed to the risk factor but the case do not. A p value < 0.05 was considered as statistically significant.

3.6. Analytic procedures used for serum and urinary measurements

All the serum and urinary parameters were measured on Olympus auto-analyzer machine.

3.6.1. Calcium:

Method- Colorimetric, chemical end point method, for serum and urine analysis

Principle- Calcium forms a purple colored complex with O-cresol-phenolphthalein in alkaline medium. Intensity of color measured at 540 nm is directly proportional to the concentration of calcium in the sample.

3.6.2. Phosphorus

Method - Colorimetric, Chemical end point method, for serum analysis.

Principle- Inorganic phosphorus reacts with ammonium molybdate and sulphuric acid (H_2So_4) to give phospho-molybdate colored complex. Intensity

of color measured at 340 nm is directly proportional to the phosphorus concentration in the sample.

3.6.3. Uric acid

Method- Colorimetric, Enzymatic end point method, for serum and urine analysis.

Principle- Hydrogen peroxide is formed when uric acid reacts with oxygen in presence of an enzyme oxidase. Hydrogen peroxide reacts with 4-aminophenazone in a series of enzymatic reactions to give a red-violet colored product. Intensity of the color is directly proportional to the uric acid concentration in the sample.

3.6.4. Oxalate

Method- Colorimetric, Enzymatic end point method (quantitative).

Principle- Hydrogen peroxide is formed when oxalate reacts with oxygen in presence of an enzyme oxidase. Hydrogen peroxide reacts with 3-methyl-2-benzothiaezoline hydrazone and 3-dimethyl amino-benzoic acid in a series of reactions to give colored complex. Intensity of color measured at 590 nm is directly proportional to the concentration of oxalate in the sample.

3.6.5. Citrate

Method- Manual, Colorimetric, Chemical end point method.

Principle- Citrate reacts with alkaline pyridium in the presence of acetic anhydride to produce colored complex. Intensity of color measured at 428 nm is directly proportional to the concentration of citrate.

3.6.6. Magnesium

Method- Colorimetric, Chemical end point method.

Principle- Magnesium ion reacts with xylidyl blue in alkaline medium to form a purple colored compound. Intensity of the color is directly proportional to the concentration of magnesium in the sample. Calcium is excluded from the reaction by complexion with ethylene diamine tetraacetic acid (EDTA).

3.6.7. Creatinine

Method- Colorimetric, end point method.

Principle- Picrate forms red colored complex with creatinine in alkaline medium. Intensity of the color is directly proportional to the concentration of creatinine.

3.6.8. Sodium and Potassium

Method- ISE (Ion Selective Electrode)

Principle- Measurement of electro-motive force (EMF) differences between sodium, potassium electrodes and a reference electrode with constant EMF. The EMF from each electrode is directly proportional to the respective ionic concentration.

3.6.9. Bicarbonate

Method- Colorimetric, Enzymatic end point method.

Principle- Based on measurement of total Co_2 in serum sample.

3.6.10. Urinary pH

The urine pH is measured by a pH electrode.

3.6.11. Albumin and total protein

Method- Colorimetric, Chemical end point method

Principle for albumin- Bromocresol green forms colored complex with albumin in acidic medium. Principle for total protein- Proteins forms a purple colored complex with cupric ions in alkaline medium Intensity of the color is directly proportional to the concentration of albumin/ total protein due to change of absorbance in photometric measurement.

OBSERVATIONS

Four hundred and fifty urolithiasis patients from Tamilnadu were evaluated from April 2006 to January 2008. Out of them 39 matched pairs were selected from urology clinic and lithotripsy unit, using preset inclusion and exclusion criteria. These clinics examine 700 stone patients annually from all parts of India. The mean age of the cases and controls was 38.5 years (Range 18-69 years) and 37.6 years (range 18-78 years) respectively. Out of 39 matched pairs, 25 (64%) were male and 14 (36%) were females. Thirty-one cases (79%) were first time stone formers and the rest had recurrence (Figure-1).

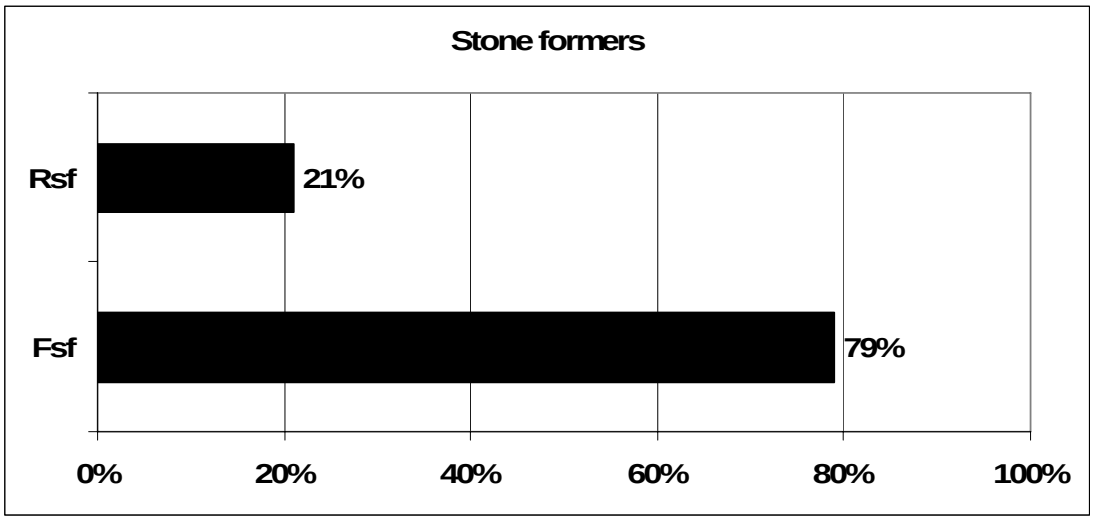

Figure-1: Figure showing percent point of First time (Fsf) and Recurrent stone formers (Rsf).

Of the eight recurrent stone formers, three had past history of recurrent calculuria.

4.1. Serum values

No significant difference was found between affected and unaffected siblings for serum calcium (p=0.18), phosphorus (p=0.10), uric acid (p=0.15), A-G ratio (p=0.51), potassium (p=0.51) and serum bicarbonate (p=0.26). Only serum sodium was found to be higher in unaffected siblings (p=0.01) (Table-1).

Table-1: Average serum parameters (continuous variables) for Cases and Controls.

Parameters	**Cases (Mean±SD)**	**Controls (Mean±SD)**	**p† value**
Calcium	9.23±0.43	9.10±0.39	0.18
Phosphorus	3.93±0.68	4.17±0.63	0.10
Uric acid	5.0±1.05	4.8±1.13	0.15
A:G ratio	1.34±0.23	1.37±0.25	0.51
Sodium	138.7±2.4	140.2±2.4	0.01
Potassium	4.24±0.42	4.19±0.32	0.51
Bicarbonate	26.0±2.30	26.5±1.77	0.26

†t-test

Blood was withdrawn for all the above mentioned parameters as an ambulatory setting.

4.2. Urine phenotyping

All of the cases and controls were found normal for ambulatory 24 hour urinary magnesium and urinary pH. All the cases were normal for urinary uric acid level but one of the controls was found to be hyperuricosuric.

4.2.1. Ambulatory urinary volume

The average urinary volume in stone formers and matched controls was 2457 and 2155 ml respectively. Urinary volume of 1500 ml or less was considered abnormally low in cases and controls. In this study, there were 12 discordant pairs (case and control had different urinary volume). There were 9 (75%) pairs where the control had low urinary volume but the case had not, and 3 (25%) pairs where the cases had low urinary volume but the control was normal for urinary volume (Table-2).

Table-2: Low and normal urinary volume among matched pairs.

		CONTROLS		TOTAL	p† -value
		Low volume	Normal volume		
CASES	Low volume n=08	05	03	**08**	0.15
	Normal volume n=31	09	22	**31**	
TOTAL		**14**	**25**	**39**	

† McNemar test

The two-tailed P value was 0.15. This difference is not statistically significant. The odds ratio is 0.33 with a 95% confidence interval extending from 0.058 to 1.336.

4.2.2. Ambulatory 24 hour urinary calcium

Out of Thirty-nine matched pairs, 8 cases were hypercalciuric and thirty-one cases were normal for urinary calcium. Of these eight cases, 5 of their matched controls were also found to be hypercalciuric. Three controls had hypercalciuria though their cases were normal for urinary calcium. In this analytical study there were 6 discordant pairs (case and control had different urinary calcium). Three (50%) pairs where the controls had hypercalciuria, the cases were normal for urinary calcium and 3 (50%) pairs where the cases had high urinary calcium the controls were normocalciuric (Table-3).

Table-3: Hypercalciuria and normal urinary calcium among matched pairs.

		CONTROLS		TOTAL	p† -value
		Hypercalciuria	Normal urinary calcium		
CASES	Hypercalciuria n=08	05	03	**08**	0.68
	Normal urinary calcium n=31	03	28	**31**	
TOTAL		**08**	**31**	**39**	

† McNemar test

The two-tailed P value was 0.68; this difference is not statistically significant.

4.2.3. Ambulatory 24 hour urinary oxalate

Five cases had hyperoxaluria and high urinary oxalate was also seen in 2 of their matched controls. Three controls had hyperoxaluria though their cases were normal for urinary oxalate (Table-4).

Table-4: Hyperoxaluria and normal urinary oxalate among matched pairs.

		CONTROLS		TOTAL	p†-value
		Hyperoxaluria	Normal urinary oxalate		
CASES	Hyperoxaluria n=05	02	03	**05**	0.68
	Normal urinary oxalate n=34	03	31	**34**	
TOTAL		**05**	**34**	**39**	

† McNemar test

Similar to the urinary calcium, there were 6 discordant pairs (cases and controls had different urinary oxalate), 3 (50%) pairs where the controls had hyperoxaluria but the cases were normal and 3 (50%) pairs where the cases were hyperoxaluric but the controls were normal for urinary oxalate. The two-tailed P value was 0.68 which was not statistically significant.

4.2.4. Ambulatory 24 hour urinary citrate

Out of Thirty-nine matched pairs, 9 cases were hypocitraturic and thirty cases were normal for urinary citrate. Of these 9 cases, 4 of their matched controls were also found to have hypocitraturia. Two controls had

hypocitraturia though their cases were normal for urinary citrate. In this study there were 7 discordant pairs. There were 5 (71%) pairs where the cases were exposed to the risk factor (hypocitraturia) but the controls were normal for urinary citrate and 2 (29%) pairs where the controls had low urinary citrate but cases were normal (Table-5).

Table-5: Hypocitraturia and normal urinary citrate among matched pairs

		CONTROLS		TOTAL	p† -value
		Hypocitraturia	Normal urinary citrate		
CASES	Hypocitraturia n=09	04	05	**09**	0.45
	Normal urinary citrate n=30	02	28	**30**	
TOTAL		**06**	**33**	**39**	

† McNemar test

The two-tailed P value was not statistically significant (p=0.45). The odds ratio was 2.5 with a 95% confidence interval extending from 0.40 to 26.2.

DISCUSSION

Stone disease is common with a lifetime risk of stone formation exceeding 12% in men and 6% in women.[30,23] The prevalence appears to have increased for men and women in the last quarter of the twentieth century.[23] Curhan GC et.al[29] corroborated this apparent increase may be due to an actual increase in stone disease or it may stem from increased detection of asymptomatic stones with the greater use and higher sensitivity of imaging studies. Lieske JC et.al.[128] found that the incidence rates since 1990 is falling in men and have reached a plateau in women. Reports also suggest that the likely hood of forming another stone after the first episode is about 30-40% in 5 years.[3,6]

Curhan GC et.al[14] found that the risk of becoming a stone former is more than 2.5 times in individuals with a family history of stone disease. A family history increases the risk of kidney stone passage independent of dietary risk factors.[31] This higher risk is likely due to a combination of genetic predisposition as well as similar environmental exposures.[29] Identification and characterization of families of recurrent stone formers is essential for the identification of unique genetic, environmental and metabolic factors that predispose individuals to recurrent calcium oxalate stone formation. While a number of genetic factors have been clearly associated with rare forms of nephrolithiasis, the information regarding genetic contribution for the common forms of stone disease is still limited.

In 1975, Pak et.al.[5] developed a simple protocol in 1978 to identify the underlying cause of Urolithiasis. This protocol disclosed a physiologic abnormality in nearly 90% of cases and provided a definitive diagnosis in 95% of patients.[3,4] This ambulatory instrument made diagnostic separation and classification of urolithiasis more accessible and more practical for all patients. The ability to distinguish among the underlying physiologic disturbances allowed the application of a selective treatment programme on the basis of correction of the specific physiologic derangement.

With multifactorial etiology, a urinary risk factor which would reliably predict the likelihood of stone recurrence in the patient with upper urinary tract stones would help the clinician to select appropriate preventative therapy. Observational studies have shown urinary parameters as a risk factor and a beneficial effect of dietary modification in them. Studies failed to show that there is a difference in dietary intake between stone formers and controls from the same geographic area.[129]

In our study, no statistical difference was found between cases and their matched controls for 24-hour urinary calcium, oxalate and citrate and urinary volume. Literature also shows that individual urinary risk factors do not reliably predict the subsequent course of stone disease.[130,131] Thus none of the indices developed to date combines easy applicability in usual clinical settings with sufficient predictive power to be useful to the clinician in making treatment decisions.

The pathogenesis of stones was explained principally on the basis of formation of crystals which result from supersaturation of urine with stone forming salts. If there are no crystals—there are no stones. The hypothesis had the attraction of simplicity. It has been said ‘‘given two or three equally predictive theories, choose the simpler theory’’ (Occam's razor).[132] Furthermore, it is easy to measure the supersaturation of urine and make attempts to predict the risk of stone formation and formulate a treatment plan, be it dietary or medicinal. This theory, however, has not completely explained all the facets of stone disease and other theories have emerged in the past decade or so.

Whilst the supersaturation theory is simple and attractive, in the more common type of stone namely calcium oxalate, the differences in terms of supersaturation between stone formers and those that do not, was not found to be significant.[133] Perhaps heterogeneous nucleation in urine which is in metastable limit, renal tubular damage with cellular dysfunction and macromolecules are more important than urine in a supersaturated state.[134] For the same reason some patients continue to form stones even after successful treatment of urinary abnormalities.[134]

SUMMARY

Despite intensive studies in the last decades many aspects of nephrolithiasis still remain to be elucidated. Supersaturation with respect to lithogenic substances explains stones composed of cystine, uric acid, struvite, and calcium stones secondary to systemic diseases. In this subset there is a clear separation between patients and controls, and stone activity is well related to alterations in the physicochemistry of the urine environment. The understanding of the mechanisms of idiopathic calcium nephrolithiasis, on the other hand is controversial, because we are still unable to establish clear-cut cause-effect relationship between metabolic and physicochemical abnormalities and stone formation. Recent studies have been centered on the kidney; not only as the end organ of urometabolic derangements due to systemic or environmental factors, but also as a complex laboratory where some events conducive to (Renal tubular damage and cellular dysfunction)[121] and others protect (macromolecules)[122] from lithogenesis. Many of these phenomena occur in the proximal tubule.

Molecular biology has explained some types of hypercalciuria, which are due to genetic mutations altering tubular function and similar results are expected for hypocitraturia and hyperoxaluria. The latter is conducive to stone formation through several mechanisms including supersaturation, oxidative stress on tubular cells, and interference with some natural inhibitors. The long list of inhibitors includes ionic and macromolecular moieties, some being produced within the nephron in response to lithogenic insults and some affecting not only

crystallization but also crystal cell adherence. Crystal trapping is believed to anticipate a renal stone. However, much has still to be clarified on their actual role in calcium nephrolithiasis, by what mechanisms they act, if patients and controls differ in the excretion and structure of some inhibitors, and whether differences are genetically determined.

Debate such as this has long been overdue. It is entirely possible that none of the above is the key to stone formation but is the final stage of a process that was initiated by something else which may be the real key.

CONCLUSION

We conducted this study with a research question; do different people living in the same environment consuming the same diet have different urinary profile? We found urinary profile in family members of a stone patient is similar. It appears some other factor must have been present in stone formers and will require further research. At present the usefulness of uro-metabolic evaluation and subsequent therapy advice appears to have little value.

LIMITATIONS

1. Diet was retrospectively assessed, so their may be chances of recall bias.
2. Sample size was small.

Bibliography

1. Blacklock NJ. Renal stone research symposium. London, Churchill Livingstone.1968.p2-3.
2. Coe FL, Keck J, Norton E. The natural history of untreated calcium nephrolithiasis. JAMA 1997;238:1519.
3. Borghi L, Schianchi T, Meschi T, et al. Comparison of two diets for the prevention of recurrent stones in idiopathic hypercalciuria. N Engl J Med 2002;346:77-84.
4. Uribarri J, Oh MS, Carrol HJ. The first kidney stone. Ann Intern Med.1989;111:1006-9.
5. Pak CYC, Kaplan RA, Bone H, *et al:* A simple test for the diagnosis of absorptive, resorptive and renal hypercalciurias.N Engl J Med 1975;292:497-500.
6. Pak CYC, Fetner C, Townsend J, *et al:* Evaluation of calcium urolithiasis in ambulatory patients. Comparison of results with those of inpatient evaluation. Am J Med 1978;64:979- 987.
7. Pak CYC, Britton F, Peterson R, *et al:* Ambulatory evaluation of nephrolithiasis. Classification, clinical presentation and diagnostic criteria. Am J Med 1980;69:19-30.
8. Verbaeys A, Minnaert H, de Paepe M, *et al:* Results of uro-metabolic evaluation in 127 patients with renal calculous disease. Urology 1985;25:22-5.
9. Churchill DN: Medical treatment to prevent recurrent calcium urolithiasis. Miner Electrolyte Metab 1987;13:294-304.
10. Ljunghall S, Fellstrom B, and Johansson G: Prevention of renal stones by a high fluid intake? Eur Urol 1988;14:381-5.

11. Hiatt RA, Ettinger B, Caan B, *et al:* Randomized controlled trial of a low animal protein high fiber diet in the prevention of recurrent calcium oxalate kidney stones. Am J Epidemiol 1996;144:25–33.
12. Nordin BEC. Hypercalciuria. Clinical Science and Molecular Medicine. 1977;52:1.
13. Williams RE. Long term survey of 538 patients with upper urinary stone. BJU; 1963;35:416-8.
14. Curhan G, Willett W, Rimm E, et al. Family history and risk of kidney stones. J Am.Soc.Nephrol.1997;8:1568-73.
15. Ettinger B, Pak CY, Citron JT, et al. Potassium-magnesium citrate is an effective prophylaxis against recurrent calcium oxalate nephrolithiasis. J Urol 1997;158:2069-73.
16. Ettinger B, Tang A, Citron JT, et al. Randomized trial of allopurinol in the prevention of calcium oxalate calculi. N Engl J Med 1986;315:1386-9.
17. Ettinger B, Citron JT, Livermore B, et al. Chlorthalidone reduces calcium oxalate calculous recurrence but magnesium hydroxide does not. J Urol 1988;139:679-84
18. Takashi Y, Paramjit SC, Fan J. Comparison of comprehensive and limited metabolic evaluations in the treatment of patients with recurrent calcium urolithiasis. J Urol;1999:161:1449-52.
19. Norma RW. Metabolic evaluation of stone disease patients: a practical approach. Curr opin Urol.2001;11:347-51.
20. Siener R et.al.The efficacy of dietary intervention on urinary risk factors for stone formation in recurrent calcium oxalate stone patients. J Urol.2005;173:1601-5.
21. Margerat SP, Lotan Y.Urinary lithiasis:Etiology,Epidemiology and Pathogenesis.Campbell-walsh urology. Alan JW et.al.(edt).2007;9th edtion:2:p1363.
22. Johnson CM,Wilson DM, O'Fallon WM, et al. Renal stone epidemiology: a 25-year study in Rochester, Minnesota. Kidney Int 1979;16:624-31.

23. Stamatelou KK, Francis ME, Jones CA, et al. Time trends in reported prevalence of kidney stones in the United States: 1976–1994. Kidney Int 2003;63:1817-23.
24. Stamatelou kk et.al. Time trends in reported prevalence of kidney stones in United States:1976-1994. Kidney Int.2003;63:1817-23.
25. Norlin A, Lindell B,Granberg PO. Urolithiasis: a study of its frequency. Scand.J.Urol.Neph.1976;10:150-3.
26. Yoshida and Okada Y. Epidemiology of Urolithiasis in Japan: A chronological and geographical study. Urol.Int;1990:45:104-11.
27. Serio A, Fraioli A. Epidimiology of Urolithiasis. Nephron.1999:81:S1:26-30.
28. Trinchieri et al. Increase in prevalence of symptomatic upper urinary tract stones during the last ten years. Eur.Urol.2000;37:23-5.
29. Curhan GC. Epidemiology of Stone Disease.UCNA; 2007;34:287-93.
30. Johnson CM,Wilson DM, O'FallonWM, et al. Renal stone epidemiology: a 25-year study in Rochester, Minnesota. Kidney Int 1979;16:624-31.
31. Drongelan JV et.al. Impact of Urometabolic evaluation on prevention of Urolithiasis: a retrospective study.Kidney Int.2001;60:3:1141-3.
32. Resnick M, Pridgen DB, Goodman HO. Genetic predisposition to formation of calcium oxalate renal calculi. N Engl J Med 1968;278:1313-8.
33. Atan L, Andreoni C, Ortiz V, et al. High kidney stone risk in men working in steel industry at hot temperatures. Urology 2005;65:858-61.
34. Prince CL, Scardino PL, Wolan CT. The effect of temperature, humidity, and dehydration on the formation of renal calculi. J.Urol;1956:75:209-15.
35. D'Angelo A, Calo L, Cantaro S, et al. Calciotropic hormones and nephrolithiasis. Miner Electrolyte Metab 1997;23:269-72.
36. Taylor EN, Stampfer MJ, Curhan GC. Obesity, weight gain, and the risk of kidney stones. JAMA 2005;293:455-62.
37. .Kramer HM, Curhan G. The association between gout and nephrolithiasis: the National Health and Nutrition Examination Survey III, 1988–1994. Am J Kidney Dis 2002;40:37-42.

38. Kramer HJ, Choi HK, Atkinson K, et al. The association between gout and nephrolithiasis in men: The Health Professionals' Follow-Up Study. Kidney Int 2003;64:1022-6.
39. Taylor EN, Stampfer MJ, Curhan GC. Diabetes mellitus and the risk of nephrolithiasis. Kidney Int 2005;68:1230-5.
40. H.G. Tiselius, D. Ackermann, P. Alken, C. Buck, P. Conort, M. Gallucci. Guidelines on Urolithiasis. Eur Urol 2001;40:362-371.
41. Robertson WG, Peacock M. The pattern of urinary stone disease in Leeds and in the United Kingdom in relation to animal protein intake during the period 1960-1980. Urol.Int. 1982;37:394.
42. Goldfarb S. The role of diet in pathogenesis and therapy of nephrolithiasis. Endocrinl.Metab.Clin.N.Am.1990;19:p805-20
43. Hosking DH, Erickson SB, Van Den Berg CJ, Wilson DH, Smith LH. The stone clinic effect in patients with idiopathic calcium urolithiasis. J Urol.1983; 130:1115-1118
44. Robertson WG, Peacock M, Hodgkinson A. Dietary changes and the incidence of urinary calculi in the U.K. between 1958 and 1976. J Chronic Dis.1979;32:469-76.
45. Larsson L, Tiselius HG. Hyperoxaluria. Miner Electrolyte Metab 1987;13:242-50.
46. Muldowney FP, Freaney R, Moloney MF. Importance of dietary sodium in the hypercalciuria syndrome.Kidney Int 1982;22:292-6.
47. Lemann J Jr, Piering WF, Lennon EJ. Possible role of carbohydrate-induced calciuria in calcium oxalate kidney-stone formation. N Engl J Med 1969;280:232-7.
48. Johansson G, BackmanU, Danielson BG, et al. Biochemical and clinical effects of the prophylactic treatment of renal calcium stones with magnesium hydroxide. J Urol 1980;124:770-4.
49. Lemann J Jr, Pleuss JA, Gray RW, et al. Potassium administration reduces and potassium deprivation increases urinary calcium excretion in healthy adults Kidney Int 1991;39:973-83.

50. Albright F, Henneman P, Benedict PH, Forbes AP. Idiopathic hypercalciuria (a preliminary report). Proc R Soc Med 1953;46:1077-81.
51. Pak CYC, Ohata M, Lawrence EC, Snyder W. The hypercalciurias: causes, parathyroid functions and diagnostic criteria. J Clin Invest 1974;54:387-400.
52. Coe FL, Bushinsky D. Pathophysiology of idiopathic hypercalciuria. Am J Physiol 1984;247:1-13.
53. Hess B, Lippuner K, Takkinen R, Villiger L, Ackermann D, Jaeger Ph. Effect of oral calcium loading in intact PTH and calcitriol in idiopathic renal calcium stone formers and healthy controls. Nephrol Dial Transplant 1995;10:1614-9.
54. Hess B. Nutritional aspects of stone disease. Endocrinol. Metab. Clin. N.Am. 2002;31:p1021.
55. Curhan G, Willett W, Speizer F, et al. Comparison of dietary calcium with supplemental calcium and other nutrients as factors affecting the risk for kidney stones in women. Ann Intern Med 1997;126:497-504.
56. Curhan GC, Willett WC, Knight EL, et al. Dietary factors and the risk of incident kidney stones in younger women (Nurses' Health Study II). Arch Intern Med 2004;164:885-91.
57. Taylor EN, Stampfer MJ, Curhan GC. Dietary factors and the risk of incident kidney stones in men: new insights after 14 years of follow-up. J Am Soc. Nephrol 2004;15:3225-32.
58. Bataille P, Charransol G, Gregoire I, et al. Effect of calcium restriction on renal excretion of oxalate and the probability of stones in the various pathophysiological groups with calcium stones. J Urol 1983;130:2:218-23.
59. Coe FL, Favus MJ, Crockett T. et.al. Effects of low calcium diet on urine excretion, parathyroid function and serum 1,25$(OH)_2D_3$ levels in patients with idiopathic hypercalcium and in normal subjects.AmJ.Med.1982;72:25-32.
60. Hess B. Low calcium diet in hypercalciuric calcium nephrolithiasis: first do no harm. Scanning Microsc.1996;10:547-56.

61. Curhan GC, Willett WC, Rimm EB, et al. A prospective study of dietary calcium and other nutrients and the risk of symptomatic kidney stones. N Engl J Med 1993;328:833-8.
62. Jackson RD, LaCroix AZ, Gass M, et al. Calcium plus vitamin D supplementation and the risk of fractures. N Engl J Med 2006;354:7:669-83.
63. Zarembski PM, Hodgkinson A. Some factors influencing the urinary excretion of oxalic acid in men. Clin Chim Acta 1969;25:1-10.
64. Larsson L, Tiselius HG: Hyperoxaluria: Miner Electrolyte Metab.1987;13:242.
65. Dobbins JW, Binder HS. Effect of bile salts and fatty acidson the colonic absorption of oxalate. Gastroenterology.1976;70:p1096.
66. Holmes RP, Assimos DG. The impact of dietary oxalate on kidney stone formation. Urol Res 2004;32:5:311-6.
67. Kasidas GP, Rose GA. Oxalate content of some common foods; determination by enzymatic method. J Hum.Nutr.1980;34:p2555.
68. Robertson WG, HughesH. Importance of mild hyperoxaluria in the pathogenesis of urolithiasis- new evidence from studies in the Arabian peninsula. Scanning Microsc.1993;7:391-401.
69. Hess B, Jost C, zipperle L et.al. High calcium intake abolishes hyperoxaluria and reduces urinary crystallization during a 20-fold normal oxalate load in humans. Nephrol Dial Transplant.1998;13:2241-7.
70. Holmes RP, Goodman HO, Assimos DG. Contrbutions of dietary oxalate to urinary oxalate excretion. Kidney Int.2001;59:270-6.
71. Curhan GC, Curhan SG. Diet and urinary stone disease. Curr.Opin.Urol.1997;7:222-5.
72. Pak CYC, Peterson R, Poindexter. Adequacy of a single stone risk analysis in the medical evaluation of Urolithiasis.J.Urol.2001;165:378-381.
73. Siener R, Honow R, Voss S, et al. Oxalate content of cereals and cereal products. J Agric Food Chem 2006;54:8:3008-11.

74. Holmes R, Kennedy M. Estimation of the oxalate content of foods and daily oxalate intake. Kidney Int 2000;57:1662-7.
75. Anderson DA. Historical and geographical differences in the pattern of incidence of urinary stones considered in relation to possible etiological factors. In Hodgkinson A, Nordin BEC (eds): Proceeding of the Renal Stone Research Symposium.1969,pp7
76. Anderson DA. Environmental factors in the etiology of urolithiasis. In Civuentes-Delatte A, Rapado A, Hodgkinson A et.al. (eds) Urinary calculi.1973,p130.
77. Fellstrom B, Danielson BG, Karlstorm B et.al. Dietary history and dietary records in renal stone patients and controls. In Schwille PO, Smith LH, Robertson WG et.al. (eds) Urolithiasis and Related Clinical Research.1985. pp.73.
78. Wasserstein AG, Stolley PD, Soper KA. et.al. Case control stuey of risk factors for idiopathic calcium nephrolithiasis. Miner. Electrolyte. Metab.1987; 13:p85.
79. Breslau NA, Brinkley L, Hill KD, Pak CYC. Relationship of animal protein-rich diet to kidney stone formation. J Clin Endocrinol Metab 1988;66:140-6.
80. Holmes RP, Goodman HO, Hart LJ, Assimos DJ. Relationship of protein intake to urinary oxalate and glycolate excretion. Kidney Int1993;44:366-372.
81. Lemieux G Kiss A-L, Lemieux C et.al. Renal tubular biochemistry during acute and chronic metabolic alkalosis in the dog. Kidney Int.1986;27;743.
82. Giannini S, Nobile M, Sartori L et al. Acute effects of moderate dietary protein restriction in patients with idiopathic hypercalciuria and calcium nephrolithiasis. Am J Clin Nutr 1999;69:267-271.
83. Robertson WG, Peacock M, Heyburn PJ et.al. Should recurrent calcium oxalate stone formers eat less animal protein? In Smith LH, Robertson WG, Finlayson B (eds). Urolithiasis: Clinical and Basic Research.1981,p.359.

84. Pak CYC. Citrate and renal calculi: new insights and future directions. Am J Kidney Dis 1991;17:420–5.
85. Erwin DT, Kok DJ, Alam J, et al. Calcium oxalate stone agglomeration reflects stoneforming activity: citrate inhibition depends on macromolecules larger than 30 kilodalton. Am J Kidney Dis 1994;24:893-900.
86. Hess B, Zipperle L, Jaeger Ph. Citrate and calcium effects on Tamm-Horsfall glycoprotein as a modifier of calcium oxalate crystal aggregation. Am J Physiol 1993;265:784-91.
87. Simpson DP. Citrate excretion: a window on renal metabolism. Am J Physiol 1983;244:223-34.
88. Hess B, Michel R. Takkinen, Ackermann D, Jaeger Ph. Risk factors for low urinary citrate in calcium nephrolithiasis: low vegetable fibre intake and low urine volume to be added to the list. Nephrol Dial Transplant 1994;9:642-9.
89. Kessler T, Hesse A. Cross-over study of the influence ob bicarbonate-rich mineral water on urinary composition on comparison with sodium potassium citrate in healthy male subjects. Br J Nutr 2000;84:865-71.
90. Wabner CL, Pak CYC. Physiological and physicochemical action of orange juice in nephrolithiasis. J Urology 1992;149:1405-8.
91. Hiatt RA, Ettinger B, Caan B, Quesenberry CP Jr, Duncan D, Citron JT. Randomized controlled trial of a low animal protein, high fiber diet in the prevention of recurrent calcium oxalate kidney stones. Am J Epidemiol 1996;144:25–33.
92. Agus ZS, Goldfarb S, Wasserstein AW: Calcium transport in nephron. Rev.Physiol. Biochem. Pharmacol. 1981;155:p90.
93. Blumenfeld JD,Vaughan ED.Renal Physiology and Pathophysiology. Walsh et.al.(edt)Campbell Urology.8thedition:Ch6:pp192.
94. Lemann JJ, Adams ND, Gray RW. Urinary calcium excretion in human beings. N Engl J Med 1979;301:535-541.

95. Massey LK, Whiting SJ. Dietary salt, urinary calcium and bone loss. J Bone Miner Res 1996;11:731-6.
96. Breslau Na, McGuire JL, Zerwekh JE, Pak CYC. The role of dietary sodium on renal excretion and intestinal absorption of calcium and on vitamin D metabolism. J Clin Endocrinol Metab 1982;55:369-373.
97. Martini LA, Szejnfeld VL, Colugnati AB, Cuppari L, Schor N, Heilberg IP. High sodium chloride intake is associated with low bone density in calcium stone forming patients. Clin Nephrol 1999:19:127-9.
98. Sakhaee K, Harvey JA, Padalino PK, Whitson P, Pak CYC. The potential role of salt abuse on the risk for kidney stone formation. J Urol 1993;150:310-2.
99. Edwards BR, Citron JT, Tang A et.al. Micropuncture study of diuretic effect on sodium and calcium re-absorption in the dog nephron. J Clin Invest.1973;52:2418.
100. Lau YK, Wasserstein AG, Westby GR et.al. Proximal tubular defects in idiopathic hypercalciuria: resistant to phosphate administration. Miner Electrolyte Metab.1982;7:p237.
101. Hughes C, Dutton S, Trusell A. High intakes of ascorbic acid and urinary oxalate. J Hum Nutr 1981;35: 274-280.
102. Wandzilak T, D'Andre S, Davis P, Williams H. Effect of high dose of vitamin C on urinary oxalate levels. J Urol 1994;151:834-7.
103. Curhan GC, Willett WC, Speizer FE et.al.Intake of vitamins B6 and C and the risk of kidney stones in women. J Am Soc Nephrol 1999;10:840-5.
104. Traxer O, Huet B, Poindexter J, et al. Effect of ascorbic acid consumption on urinary stone risk factors. J Urol 2003;170:397-401.
105. Curhan GC, Willett WC, Rimm EB, et al. A prospective study of the intake of vitamins C and B6, and the risk of kidney stones in men. J Urol 1996;155:6:1847-51.
106. Frank M, DeVries A, Lazebnik J et.al. Epidemilogical investigations of Uroloithiasis in Israel. J Urol.1959;81:497-504:
107. Borghi L, Meschi T, Amato F, et al. Urinary volume, water and

recurrences in idiopathic calcium nephrolithiasis: a 5-year randomized prospective study. J Urol1996;155:839-43.

108. Agreste AS, Schor N, Heilberg IP. The effect of hardness of drinking water upon calculi growth in rats. In: Borghi L, Meschi T, Briganti A, Schianchi T, Novarini A eds, Kidney Stones (Proceedings of the 8th European Symposium on Urolithiasis).1999:p487.
109. Caudarella R, Rizzoli E, Buffa A, Bottura A, Stefoni S. Comparative study of the influence of 3 types of mineral water in patients with idiopathic calcium lithiasis. J Urol.1998; 159:658-663.
110. Agreste AS, Schor N, Heilberg IP. Mineral composition of natural (NW) and sparkling water (SW) and calculi growth in rats. In: Borghi L, Meschi T, Briganti A, Schianchi T, Novarini A (eds). Kidney Stones (Proceedings of the 8th European Symposium on Urolithiasis). 1999:p489.
111. Marangella M, Vitale C, Petrarulo M, Rovera L, Dutto F. Effects of mineral composition of drinking water on risk for stone formation and bone metabolism in idiopathic calcium nephrolithiasis. Clin Sci 1996;91:313–318.
112. Rodgers AL. Effect of mineral water containing calcium and magnesium on calcium oxalate urolithiasis risk factors. Urol Int 1997;58:93–99.
113. Pak CYC, Sakhaee K Crowther C et.al. An Objective evidence for the beneficial effect of a high fluid intake in the management of nephrolithiasis. Ann.Intern.Med.1980;93:36.
114. Curhan GC, Willett WC, Rimm EB, et al. Prospective study of beverage use and the risk of kidney stones. Am J Epidemiol.996;143:3:240–7.
115. Curhan GC, Willett WC, Speizer FE, et al. Beverage use and risk for kidney stones in women. Ann Intern Med 1998;128:7:534–40.

116. Wabner C, Pak C. Effect of orange juice consumption on urinary stone risk factors. J Urol.1993;149:1405–9.
117.Chandhoke PS. Evaluation of the Recurrent Stone Former. UCNA;2007:34:p320.

118.Margerat SP, Lotan Y.Urinary lithiasis:Etiology,Epidemiology and Pathogenesis.Campbell-walsh urology. Alan JW et.al.(edt).2007;9th edtion:2:1365-70.
119.Kok DJ. Clinical implications of physicochemistry of stone formation. Endocrinol Metab Clin N Am.2002;31:856–867.
120. Kok DJ, Khan SR. Calcium oxalate Nephrolithiasis, a free or fixed particle disease. Kidney Int.1994;46:847–54.
121. Finlayson B, Reid F. The expectation of free and fixed particles in urinary stone disease. Invest Urol.1978;15:442–8.
122.Randall A (1940) The etiology of primary renal calculus. Int Abstr Surg 71:p209.
123. Khan SR.Renal tubular damage/dysfunction: key to the formation of kidney stones Urol. Res.2006;34:86-91
124.Ryall RL, Grover PK Thurgood LA et.al. The importance of a clean face: the effect of different washing procedures on the association of Tamm–Horsfall glycoprotein and other urinary proteins with calcium oxalate crystals. Urol Res.2007;35:1-7.
125.Tiselius HG, Hallin A, Lindback B. Crystallisation properties in stone forming and normal subjects' urine diluted using a standardised procedure to match the composition of urine in the distal part of the distal tubule and the middle part of the collecting duct. Urol Res.2001;29:75-82.
126.Nakagawa et al,Isolation from human calcium oxalate renal stone of nephrocalcin; a glycoprotein inhibitor of calcium oxalate crystal growth: evidence that nephrocalcin from patients with calcium oxalate nephrolithiasis is deficient in gamma-carboxyglutamic acid.J clin

invest.1987;79:1782-7.
127.Asplin et.al.Contribution of human uropontin to inhibition of calcium oxalate crystallization. Kidney Int.1998;53:194-9.
128.Lieske JC, Pena de la Vega LS, Slezak JM, et al. Renal stone epidemiology in Rochester, Minnesota: an update. Kidney Int 2006;69:4:760-4.
129.Fellstrom B, Danielson BG, Karlstrom B, et.al.: Dietary history and dietary records in renal stone patients and controls. In Schwille PO, Smith LH, Robertson WG, et.al. (eds): Urolithiasis and related clinical research.New York,Plenum;1985:p73.
130.Trinchieri A, Ostini F, Nespoli R, Rovera F, Montanari E, Zanetti G. A prospective study of recurrence rate and risk factors for recurrence after a first renal stone. J Urol;1999:162:27.
131.Curhan GC, Willett WC, Speizer FE, Stampfer MJ. Twenty-four-hour urine chemistries and the risk of kidney stones among women and men. Kidney Int;2001:59:2290.
132. W. M. Thorburn, "Occam's razor".Mind.1915;24:287-8.
133. Kavanagh J.P.Supersaturation and renal precipitation: the key to stone formation? Urol Res.2006;34:81-5.
134. Rao PN. Debate:The key to stone formation. Urol Res.2006;34:79–80.

Annexure-i

Consent Form

Urinary Biochemical Profile in Urolithiasis: A case control study from Tamil Nadu

Information to the patient:

Urinary stone disease recurs at 10% at 1 year 50% at 5 years and 70% at 10 years. We do not know why it recurs in most people. We plan to do this research on you and one of your relatives. The relative should be of the same sex, taking the same diet and living together for the last five years. The purpose of the study is to identify if there is any differences in biochemical profile between you and your family member. In this study, there is no risk for you or your family member. Infact it gives an opportunity to screen your family member for urinary stone disease. If stone disease is diagnosed in your relative, he/she will need treatment at his/her own cost.

Signature of Patient

Name…….

H. No……..

Signature of Relative (control)

Name…….

H.No……...

Relation……..

Signature of principal Investigator

Name: Dr Gaurav Gupta

Date…….

Annexure-ii

History Performa for Case and Control

Name_____Age_____Sex_____

No of family members in the family_____Male_____Female_____

How many members living with the patient for >5yrs _____

Patient is First stone former (Fsf.)_____ Recurrent stone former (Rsf.)_____

Renal / Ureteric stone / Both_____

Past Intervention for stone_____

Dates: Ist stone_____IInd stone_____

Residual after intervention_____

Medical therapy for Urolithiasis_____

Past history of Urolithiasis/ Calculuria for controls_____

Radiology: suggestive of structural abnormality_____

Medical history of

1. Gout_____2. Intestinal disease (MAS) _____

3.UTI's_____4. Fracture_____ 5. Diabetes_____

6. Bone disease_____ 7. Resection of small bowel_____

8. Medications (Steroids; Calcium supplements; vit D and C; Frusemide; triemterine; Indinavir; Sulfonamides;antacids)_____
_____

9. RTA_____10 Hyperperathyroidism_____

11. Sarcoidosis_____12. Disseminated malignancy_____

Annexure-iii

WORK SHEET

Name (Pt)_____ Name (C)_____

H.No_____ H.No._____

Address_____ Address_____

Age_____ Sex_____ Age_____ Sex_____

Serum

Ca _____ Ca _____

Po_4_____ Po_4 _____

UA_____ UA_____

Na _____ Na_____

K _____ K_____

HCo3_____ HCo3 _____

AG ratio_____ AG ratio _____

24 hr urinary

pH_____ pH_____

Ca_____ Ca_____

Oxalate_____ Oxalate_____

Uric Acid_____ Uric Acid_____

Citrate_____ Citrate_____

Magnesium_____ Magnesium_____

Creatinine_____ Creatinine_____

X-Ray KUB_____

Site of stone_____ USG-KUB _____

Name	H.no	Contro	Age	Sex	S.Ca	S.Po4	S.UA	S.Na	S.K	S.HCo3
Soma	953300C	Co	25	M	9.5	3.7	4.8	137	3.7	27
Sayed Ibrah	010167C	Co	33	M	9.6	3.5	5.2	139	4	26
Sivagami	986159C	Co	47	F	8.6	4.2	4.5	138	3.9	25
Anand	251414C	Co	34	M	9.4	3.3	5.3	144	4.2	28
Arun kumar	956897C	Co	18	M	9.4	5	5.1	139	4.1	28
Bharathi	964004C	Co	23	M	9.1	5.5	5.5	137	4	28
Arivazhagar	971618C	Co	42	M	9	3.9	3.9	138	4.1	25
Parijathan	973101C	Co	53	M	8.8	4.5	6.2	137	3.9	28
Archana	479830C	Co	20	F	9.3	4.7	2.6	143	4.3	26
Sujatha	492085B	Co	32	F	8.6	3.7	2.5	137	4.1	19
Kamalamma	514064C	Co	73	F	9.3	4.5	4.9	141	4.3	28
Sumathi	017837C	Co	38	F	9	4.9	3.7	141	4	25
Nirmala	045724D	Co	56	F	8.7	4	5.5	143	4.6	28
Shyamala	538503B	Co	32	F	9.2	3.2	4.6	145	3.5	25
Balan	042850D	Co	38	M	8.8	4.3	7.1	144	5	29
Mangalaraj	058097C	Co	48	M	8.6	4.5	6.4	143	4.1	26
Uma	056477D	Co	25	F	8.6	3.8	3.2	140	4.1	27
Sumithra	983031C	Co	28	F	9.7	4	4	139	4.7	25
Muniappan	064150D	Co	22	M	8.6	4.2	4.5	138	3.9	25
Anand V	251414C	Co	34	M	9.4	3.3	5.3	144	4.2	28
Shakeela	143701D	Co	20	F	9.2	4.6	2.7	140	4.1	26
Andrews	413654(C	Co	60	M	9.2	3.8	5.9	144	4.1	26
Settu	146964D	Co	35	M	9.6	3.6	5.6	141	5.2	27
Elumalai	111053D	Co	18	M	9.6	5.8	5.7	140	4.1	28
Gajapathy	088354D	Co	23	M	9.4	4.3	4.2	140	4	26
Sarvanan	095210D	Co	36	M	8.9	5.1	5.7	136	4.2	27
Caroline	233298A	Co	40	F	9.1	3.5	2.5	140	4.1	26
Vasugi	418010C	Co	43	F	9	4.1	5.6	138	4.3	25
Ananthi	065941D	Co	19	F	8.5	3.5	4.2	139	4	26
Senthil K	357000C	Co	25	M	9.4	3.5	5.3	139	4.1	27
Md Iqbal	306942B	Co	48	M	9.8	4.4	4	137	4.3	26
Ravishanka	130693D	Co	35	M	8.9	4.4	6	138	4.1	29
Kanthamma	123161D	Co	55	F	9.2	4.2	2.8	144	4.1	28
Ashok K	952152C	Co	22	M	10	3.7	5.6	142	4.9	28
Md.Ali	122860D	Co	54	M	8.8	2.9	5.8	140	4.1	25
Damodhara	111225D	Co	67	M	8.9	4.7	5	141	4	28
Gopiraj M	119084D	Co	23	M	8.9	3.1	4.4	142	3.8	27
Rangaraj V	619250A	Co	47	M	9.1	4.3	5.5	142	4	27
Subramani	111054D	Co	78	M	8.5	4	4.4	140	4.7	26

S.ca:serum calcium,SPo4:serum phosphorus,S.UA.:serum uric acid,S.Na.serum sodium,S.K.:serum potassium,Hco3:serum bicarbonate,AGratio:albumin Globulin ration,UpH: urinary pH.

AGRatı	pH	U U24h	U.Ca	U,Ox.	U.UA	U.Cit	U.Mg	U.Cr
1.48	6	3550	316	40	420	828	142	984
1.43	6	1425	139	20	530	2186	107	1500
1.1	5.5	950	120	14	380	195	44	608
1.4	5.5	1680	62	23	538	593	68	593
1.4	5	4630	264	50	407	1314	133	1400
1.56	5	1100	147	20	550	573	4	1200
1.35	6	5500	400	15	512	651	161	1279
1.17	5.5	2770	197	20	332	487	84	1290
1.48	6	980	131	26	461	1130	93	1000
1	6	670	129	18	359	1107	100	590
1.22	5	1030	147	34	216	698	128	855
1.19	6	4120	52	15	288	255	98	577
1.25	7	3000	123	19	570	503	90	840
1.15		1000	178	21	400	450	46	903
1.35	5.5	600	86	38.3	660	574	92	1100
1.28	5	3190	112	69	514	275	48	1500
1.16	5	1180	55	23	177	967	52	531
1.29	5	900	170	12	441	640	6	720
1.1	5.5	1800	120	14	380	195	4.5	608
	5.5	1680	62	23	538	593	68	1400
1.36	7	1170	64	20	234	167	25	386
1.2	8	1980	275	9.4	372	752	24	1100
1.63	7	3335	119	11	391	1243	141	686
1.63	7	1285	40	17	513	930	201	731
2.21	7	1685	175	11	349	301	53	996
1.46	6	1260	53	29	403	669	55	900
1.28	6	1000	102	25	304	590	10	697
	6	1346	255	31	468	1005	74	611
0.82	6	1940	175	12	330	208	76	543
1.85	7	1160	101	45	360	597	93	1000
1.36	5	2790	112	12	290	377	56	474
1.48	5	3360	119	35	560	1723	126	1300
1.17	6	1900	112	45	500	380	9	900
1.59	6	2210	31	5.6	400	173	81	624
1.28	5	4755	412	15	747	84	144	1400
1.53	7	2860	179	18	361	434	136	706
1.46	7	2120	240	22	400	892	107	1100
1.64	5.5	2600	121	27	494	671	73	1200
1.09	6	1220	52	14	309	386	58	514

S.ca:serum calcium,SPo4:serum phosphorus,S.UA.:serum uric acid,S.Na.serum sodium,S.K.:serum potassium,Hco3:serum bicarbonate,AGratio:albumin Globulin ration,UpH: urinary pH.